0993-9845

LC-85B SPECTROPHOTOMETRIC DETECTOR FOR LIQUID CHROMATOGRAPHY

0271-0050, 117V, 60 Hz Detector 8 µl Cell
0271-0051, 230V, 50 Hz Detector 8 µl Cell
0271-0033, 117V, 60 Hz Detector 1.4 µl Cell
0271-0034, 230V, 50 Hz Detector 1.4 µl Cell

Important: This equipment generates, uses, and can radiate radio frequency energy, and if not installed and used in accordance with the instruction manual, may cause interference with radio communications. It has been tested and found to comply with the limits for a Class A computing device pursuant to Subpart J of Part 15 of FCC Rules, which are designed to provide reasonable protection against such interference when operated in a commercial environment. Operation of this equipment in a residential area is likely to cause interference, in which case the user, at his own expense, will be required to take whatever measures may be required to correct the interference.

September 1982

PERKIN-ELMER NORWALK, CONNECTICUT, U.S.A.

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1 INTRODUCTION

1.1 DESCRIPTION OF THE LC-85B

The Perkin-Elmer Model LC-85B Spectrophotometric Detector is a dual beam, variable wavelength detector, designed for use in a liquid chromatography system. The detector is a self-contained unit as shown in Figure 1-1 and houses a variable wavelength spectrophotometer, a high-pressure flow-through sample cell, and the electronics required to control the unit.

The detector permits absorbance detection in the ultraviolet-visible range (190 nm to 600 nm). The spectrophotometer is insensitive to flow noise and to changes in the refractive index of solvents.

The standard assembly has a cell volume of 8 microliters. It is used in conventional chromatography, G.P.C. and preparative chromatography. The 1.4 microliter cell assembly is used for high speed chromatography, with "microbore" and other high resolution chromatography.

The Model LC-85B can be used with any laboratory recorder having a 10 mV, 2.5mV, or 1.0 mV voltage (full scale) range.

Figure 1-1 - The LC-85B Detector

1.2 SCOPE OF THE MANUAL

This instruction manual has been provided to acquaint the user with the LC-85B Dual-Beam Spectrophotometric Detector. The manual assumes that the user has developed a working knowledge of liquid chromatography, including:

- Knowing the function of each major component in a liquid chromatography system;
- The ability to choose a column based on the type of analysis to be done;
- Understanding the principles of solvent selection and sample preparation;
- Understanding the term "UV cutoff".

Should the user require an introduction to these concepts, which is outside of the scope of these instructions it may be found in Practical Liquid Chromatography - An Introduction by R.W. Yost, L.S. Ettre and R.D. Conlon, published by the Perkin-Elmer Corporation (1980). This text is available under Perkin-Elmer P/N 0993-9656.

This manual provides relevant operating instructions for the LC-85B. For the user's convenience, an operating summary is included at the back of Section 3. Installation, routine maintenance, and service procedures are also included in these instructions.

1.3 WARNINGS

> Warning: Never look directly at the deuterium lamp when it is on. To avoid UV damage to the eyes, always wear ultraviolet opaque safety glasses whenever the cover is lifted and the lamp is on. The lamp is hot. Allow the lamp to cool completely or wear protective gloves to avoid burns when handling the lamp.

1.3.1 Electrical Hazards

> Warning: High voltage is present at certain locations in the LC-85B. Before removing any panels or lifting the cover, the LC-85B must be turned OFF and disconnected from the AC power source.

Electrical service on the LC-85B should be performed only by a qualified Perkin-Elmer Service Engineer.

1.3.2 Ground Protection

All the components of the system must share a common ground, supplied by a third wire, in the electrical supply circuit. Do not disconnect or alter this third prong on the LC-85B AC line cord.

1.3.3 Solvents

Observe care in working with solvents which are hazardous in themselves or which produce hazardous by-products.

> **Warning:** Solvent vapor levels high enough to interfere with the detector should be considered hazardous for the operator who is continuously exposed to them.

1.3.4 Solvents With Auto-Ignition Temperatures Below 110°C

Certain solvents have a temperature at which they are combustible upon contact with a heated surface even in the absence of a spark or other source of ignition. For your convenience, a list of common solvents with their auto-ignition temperatures is given below.

> **Warning:** DO NOT USE CARBON DISULFIDE OR OTHER SOLVENTS WITH AN AUTO-IGNITION TEMPERATURE BELOW 110°C.

AUTO-IGNITION TEMPERATURES OF COMMON LC SOLVENTS

SOLVENT	AUTO-IGNITION °C
carbon disulfide	100
diethyl ether	180
cyclohexane	260
hexane	261
petroleum ether (naptha)	288
pentane	309
THF	321
dioxane	366
propanol	404
iso-octane	418
ethanol	423
i-proply ether	443
dimethylformamide	445
methanol	446
isopropanol	455
methylethylketone	474
acetonitrile	524
ethyl acetate	524
toluene	536
acetone	538
benzene	562

1.4 PRECAUTIONS

1.4.1 Solvents

Solvents should be degassed and filtered to below 0.5 micrometer before use. Because the inside diameter on the 1.4 µl flowcell input tubing assembly is 0.005" (only 127 microns), proper filtering of solvents is critical.

Solvent Filtration Kits 0089-0839 (Aqueous) and 0089-0841 (Organic) are available. The solvents commonly used in the far UV are: water, acetonitrile, iso-octane (2,2,4-trimethylpentane), heptane, hexane, and pentane. (Methanol and isopropanol from some suppliers may also be suitable.) Because of this small diameter, it is also important to choose the mobile phase with respect to potential solvation of the column packing material. Users may have to try several sources because UV-absoring impurity contents vary from supplier to supplier. Generally, "distilled in glass" solvents are of suitable purity.

- Use clean solvents. All solvents must be filtered prior to use. Solvents which have been distilled in glass are highly recommended.
- Degas all aqueous and most organic solvents prior to use.
- Carefully use and store flammable solvents in closed containers.
- Check compatibility of solvent(s) with the type of column(s) being used.
- Know the relative polarity and miscibility of the solvents being used for analysis.

1.4.2 Buffers

Be careful when using buffers in conjunction with organic solvents. Never leave buffers in the system overnight. Salt crystals can form and plug the connecting tubing. Flush with water and store in methanol or isopropanol. Flush the storage solvent with water before adding buffers.

1.4.3 Corrosion

Parts of the instrument which come in direct contact with the solvent are made of 316 stainless steel (or equivalent), PTFE or glass. Some of these materials are extremely sensitive to acidic chlorides.

> Important: Do not permit acidic chlorides (those with pH less than or equal to 7) to come in contact with any of the internal portions of the instrument. Instrument corrosion or failure resulting from the use of acidic chlorides is NOT covered by the warranty.

For example, some of the internal portions of the instrument are extremely sensitive to hydrochloric acid. Several publications have described the use of "TRIZMA" buffers in ion exchange work. These are available in several forms. The acid form tris (hydroxymethyl) aminomethane HC1 (TRIS-HC1), contains hydrochloric acid and MUST NOT BE USED. The pH conditions required to duplicate most published separations can be met with the phosphoric version, Mono-Tris (Hydroxymethyl) aminomethane phosphate (TRIS-H_3PO_4).

1.4.4 Thermal Stability

Drafts contribute to thermal instability in the detector. Do not operate the LC-85B in a direct draft such as caused by an air conditioner or hot air heater. Specifications are met when operating in the range of 19°-30°C.

1.4.5 Lamp Life

Because the deuterium source has a finite life, shut off the power to the LC-85B when the detector is idle for an extended period of time, e.g., over the weekend.

1.4.6 Radio Frequency Energy

The LC-85B generates, uses and can radiate radio frequency energy, and, if not installed and used in accordance with this instruction manual, may cause interference with radio communications. It has been tested and found to comply with the limits for a Class A computing device pursuant to Subpart J of Part 15 of FCC Rules, which are designed to provide reasonable protection against such interference when operated in a commercial environment.

Operation of this equipment in a residential area is likely to cause interference, in which case the user, at his own expense, will be required to take whatever measures may be required to correct the interference.

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2 INSTALLATION

2.1 SPECIAL LABORATORY REQUIREMENTS

The dimensions of the LC-85B are shown in Figure 2-1. Prepare a sturdy bench location in a clean environment with relatively stable temperature and humidity. The weight of the LC-85B is 27.2 kg (61 lbs). The bench must support the weight of the detector as well as the weight of other items to be used in the system. Provide space at the rear of the instrument for air circulation. Provide access space to both sides of the LC-85B. The solvent line exits to the right.

2.1.1 Clean Air Systems

Observe care in working with solvents which are hazardous in themselves or produce hazardous by-products.

> **Warning:** Solvent vapor levels high enough to interfere with the detector should be considered hazardous for the operator who is continuously exposed to them.

Provide a clean air source and an appropriate exhaust system where necessary.

2.1.2 Ambient Temperature and Humidity

The detector will operate within the specifications when the ambient temperature ranges from 19° to 30° degrees Celsius. Ambient relative humidity is considered to be from 20 to 80% with no condensation.

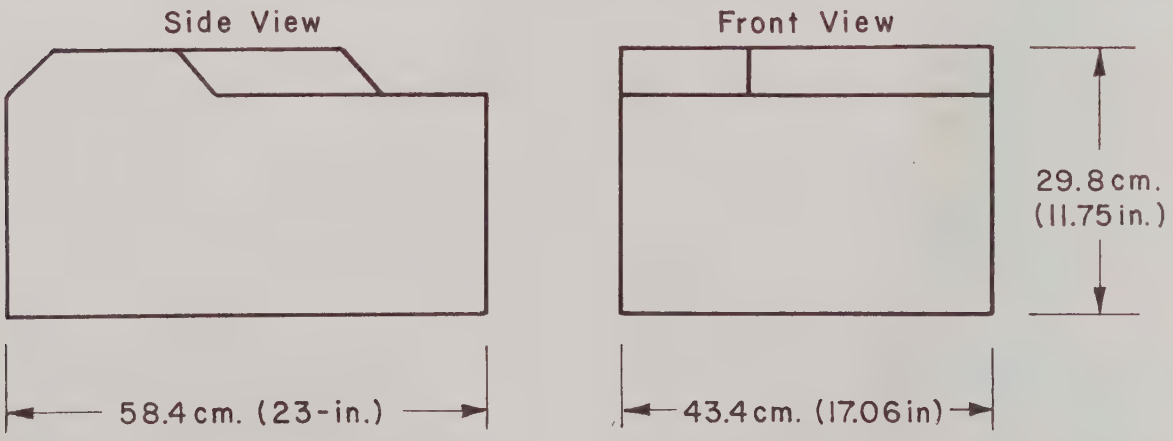


Figure 2-1 - LC-85B Dimensions

2.1.3 Electrical Requirements

The detector requires a grounded 117 VAC (nominal) or 230 VAC (nominal) source. The data processing equipment and other accessories require separate outlets. See the appropriate manuals for these power requirements.

To prevent degradation of the detector's performance the AC line voltage must remain within ±10% of the nominal voltage specified on the label located over the line cord connector on the LC-85B. To accomodate other AC line voltages, refer to Section 2.4.2.

A good earth ground is also required, common to all instrument modules and user-supplied attachments.

> Caution: The grounding must be accomplished by a third wire in the electrical supply system. Do not disconnect or alter the 3 prong ground on the 117V AC line cord.

The detector should not be attached to circuits associated with equipment of a heavy-duty nature or large or frequent loads as this may result in excessive noise which may interfere with the detector signal.

2.1.4 Gas Requirements

The detector optical paths are enclosed and purgeable with dry nitrogen to remove UV-absorbing solvent vapors. Nitrogen used for this purpose must be pure, clean, dust-free, oxygen-free and preferably boiled-off from liquid nitrogen. Pre-purified tank nitrogen is acceptable. See Section 4.6 for the correct purging procedure.

2.2 UNPACKING

2.2.1 Removal of Packing Material

Before operating this LC-85B, lift the cover and remove the following packing materials:

1. Sponge rubber securing the sheet metal baffle.
2. Six styrofoam wedges from behind the six metal clips that hold the purge cover to the optical bench.
3. The tape from behind the counter window on the cover.
4. The cardboard and metal plate from underneath the microcell cover.
5. The two tie wraps and cardboard tube from the deuterium lamp bracket.
6. The acrylic tubing covering the nuts and ferrules on the cell assembly input and output tubing.

When unpacking, check the items against the packing list. Check for any signs of obvious shipping damage. Report any damage or missing items to the carrier and to the Perkin-Elmer Corporation immediately.

2.2.2 Installation Kit

These items, included in Installation Kit 0271-0121 are shipped with the LC-85B Detector. The cell window seals, which are not used during the installation, should be stored in a clean, safe location for future use. (These are packed in plastic bags and labeled with the part number.)

P-E Part No.	Description	Quantity
0087-1130	Seal, 8 µl Cell Window	6
0271-1643	Seal, 1.4 µl Cell Window	6
0271-1298	Light Cover, Cell Adjust	1
0990-7271	Wrench, Allen 3/32	1
0087-3036	Tube, 1/16 O.D. x .015 I.D. S.S. (6 ft.)	1
0271-1441	Tube, 1/16 O.D. x .007 I.D. S.S. (20 cm.)	1
0089-1480	Tube, 1/16 O.D. x .007 I.D. S.S. (50 cm.)	1
0087-3140	Tube, Teflon 17 Gauge	1
0990-3259	Coupling, Low Dead Volume SSI	1
0087-3032	Ferrule, 1/16 CPI	1
0990-3980	Nut, 1/16 CPI Inverted Tube	1
0990-7126	Adjustable Back Pressure Regulator	1
0998-1622	Fuse, 0.5A 250 VAC	1
0998-1762	Fuse, 0.8A Slo-Blo	1
0998-1624	Fuse, 1.5A Slo-Blo	1
0271-0337	Cable Assembly, Recorder	1
0998-9845	Manual, LC-85B Detector	1

2.3 TUBING CONNECTIONS

2.3.1 General Information

Perkin-Elmer liquid chromatography systems utilize 1/8-in. O.D. tubing and fittings upstream of the pump(s) and 1/16-in. O.D. tubing and fittings after the pump(s). All of the 1/16-in tubing is #316 stainless steel or equivalent. 0.007-in. I.D. tubing should be pruchased in appropriate pre-cut lengths. 0.015-in. I.D. tubing may be cut to length if care is used. When you cut any of the 0.015-in. I.D. tubing required in your system, scribe the tubing squarely with a triangular or blade file. Then use two smooth-jaw pliers to separate the pieces of tubing. (See Figure 2-2). Gently turn the pliers in opposing directions to separate the tubing, gently bending the tube as required. The tubing ends must be filed free of burrs and snags. Use Figure 2-3 as a guide when you orient the nut and its associated ferrule(s) or collar. Note that the tubing must project past the collar or ferrule. Flush new tubing to clean before installing in the system. Tubing in Perkin-Elmer Installation kits has been internally cleaned and passivated after cutting to length at the factory.

Special care is required when preparing lengths of 0.007-in. I.D. tubing. 0.007-in. I.D. tubing is required when working with the 1.4 µl cell and high-speed columns (i.e., 3-15 cm long, 2-4.6 mm I.D., with 3-5 micron particle diameter). Tubing should be free of burrs and tested to be clear of metal particles before it is used. We recommend using only pre-cut lengths of tubing with inside diameter of 0.007-in. or smaller. In addition to the 20 and 50 cm lengths supplied with the installation kit there is also available from Perkin-Elmer a 7.5 cm length of 0.007-in. I.D. tubing (0089-1479).

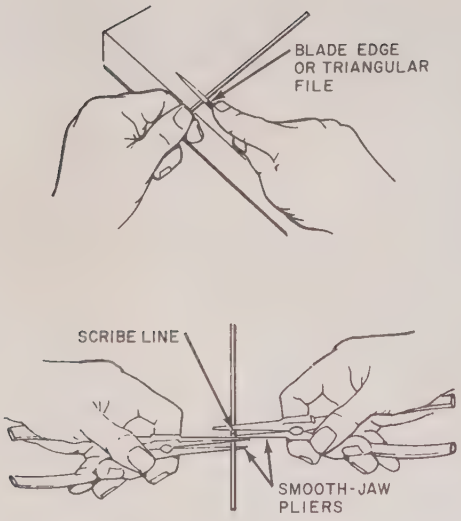


Figure 2-2 - Cutting 0.015-in. I.D. Stainless Steel Tubing

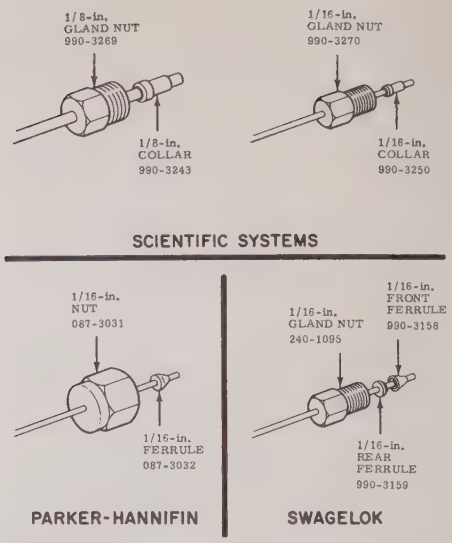


Figure 2-3 -Orientation of Hardware

When you make connections, insert the tubing and collar into the fitting seat. Press and hold the tubing to the seat bottom. Turn the nut into the fitting, carefully following the nut with the wrench. Never over-tighten the fittings. Use a wrench to tighten the nut no more than 90 degrees past fingertight when the fitting is new, 45 degrees past fingertight when the fitting is being reconnected.

Turn on the system and bring the system to its normal operating pressure after making any plumbing modifications. If leaks occur, tighten the fittings an additional 10 degrees. If the leaks persist, disassemble the affected fitting(s) and inspect the fitting(s). The threads should be in good condition and the collar or ferrule(s) should be uniformly deformed. The collar or ferrule(s) should grasp the tubing firmly. If the hardware appears to be in good condition, remake the connection, and tighten to the required level. If the leak persists, replace the hardware and repeat the procedure until leak-free operation is achieved.

2.3.2 Detector Solvent Lines – 8 µl Flowcell

The length of the tubing from the column outlet to the detector flowcell inlet should always be as short as possible. Using the two-meter (6 ft.) length of 1/16-in. O.D. x 0.015-in. I.D. stainless steel tubing (0087-3036) provided in the 85B installation kit cut a piece sufficiently long to reach from the column to the flowcell. Make certain that the tube ends are square and free of burrs. Connect the tube to the column outlet using the Parker CPI nut (0990-3980) and ferrule (0087-3032) supplied or the fittings provided with the column. Connect the other end of the tube to the SSI zero dead volume coupling (0990-3259) which is included with the installation kit and is provided with mating nuts and ferrules. Remove the nut and ferrule from the other end of the coupling and connect the flowcell inlet line which already has a mating nut and ferrule swaged to its end.

If required, the remaining tubing can be used to connect the flowcell outlet to a waste drain or to another detector. Hardware to make these connections is not provided with the 85B. If a second detector is not used we recommend that the back-pressure regulator (0990-7126) provided in the installation kit be connected to the outlet of the flowcell (Refer to Section 2.3.5).

2.3.3 Detector Solvent Lines - 1.4 µl Cell

Two lengths of 0.007-in. I.D. tubing (20 and 50 cm) are provided in the 85B installation kit for use in connecting the inlet of the 1.4 µl cell to the outlet of the column. For effective chromatography the volume of this connection should be as small as possible. Therefore, the 20 cm length of tubing (0271-1441) should be utilized in connecting the detector to the column. If the column is being operated in an oven, it may be necessary to employ the 50 cm length of tubing (0089-1480) to make a convenient connection between the two components. The tubing can be connected to the column using the parker CPI nut (0990-3980) and ferrule (0087-3032) or with the fittings supplied with the column. The 0.005-in. I.D. inlet line to the 1.4 µl flowcell is accessed via an SSI coupling clamped to the top of the optical bench casting (see Figure 2-6). The tubing from the column is connected to this inlet coupling. Use the 0.015-in. I.D. tubing when directing the outlet of the flowcell to a waste drain and a pre-finished length of 0.007-in. I.D. tubing if a second detector is to be used following the 85B.

If a second detector is not used we recommend that the back-pressure regulator (0990-7216) provided in the installation kit be connected to the outlet of the flowcell (see Section 2.3.5).

2.3.4 Nitrogen Purge Tubing

17 gauge Teflon tubing 0087-3140 is provided for purging the optics chamber with nitrogen. See the Service Section of these instructions (Section 4.6.1).

2.3.5 Back-Pressure Regulator

A back-pressure regulator (P-E Part No. 0990-7126) should be installed on the cell outlet line to increase the pressure in the flow cell to approximately 40 psi. Back-pressure reduces the formation of gas bubbles in the flowcell and will decrease any detector noise from this source. The regulator provided is adjustable from 15 to 59 psig (0.1 to 0.4 MPa) and is delivered factory set to provide a back-pressure of 40 psig (0.27 MPa) at 2 mL/min. It should be connected to the flowcell outlet with 1/16-in. O.D. by 0.015-in. I.D. stainless steel tubing. A nut and ferrule is provided with the regulator. It is recommended that the regulator be immersed in a small mouth flask of mobile phase which is utilized as the waste reservoir.

The back-pressure regulator is shipped set at 40 psi. Should adjustment be necessary, contact your Perkin-Elmer Service representative.

> Important: The total pressure on the 1.4 µl cell must not exceed 1000 psi. Cell pressure is measured directly by removing the column and measuring directly all backpressure at the desired flow rate. Cell pressure is measured indirectly by noting the backpressure with the detector removed and then replaced in the system and subtracted.

2.4 ELECTRICAL CONNECTIONS

2.4.1 Recorder and Accessory Connections

Use the recorder cable (P-E Part No. 0271-0337) provided to make the appropriate recorder connections at TB1, located on the underside of the detector cover. (see Figure 2-4 and 2-5).

The detector is shipped with the jumper in position for a 10 mV recorder. If a 2.5 mV recorder or a 1.0 mV recorder is to be used, the jumper (SP1) on the Attenuator Board must be moved (see Figure 2-5).

Recorder	Jumper Position
10 mV	AB
2.5 mV	BD
1.0 mV	BC

*If a 1V or 10 V data acquisition device will be used, use a shielded two-conductor cable or use the recorder cable to make connections for the data outputs (see Figure 2-4 and 2-5).

10V Outputs	1 V Outputs
TB1-1 Data Output	TB1-4 Data Output
TB1-2 Return	TB1-5 Return
TB1-3 Shield	TB1-6 Shield

The 1 V output has a voltage scale factor of 200 mV/Absorbance$_{1\ cm}$ unit.

The 10 V output has a voltage scale factor of 2 V/Absorbance$_{1\ cm}$ unit.

2.4.2 Fuse Insertion

First, refer to Figure 2-6 and make certain that the small printed circuit board located behind the line fuse (left side panel) is inserted to correspond to the line voltage available. Make sure this voltage corresponds with the label over the line fuse. To check the proper operating voltage range setting, remove the AC line cord from the instrument. Slide the clear plastic over over the fuse all the way to the left. Rotate the FUSE PULL to the left and remove the fuse. Pull out the printed circuit board located below the fuse and orient the printed circuit board so that the desired voltage setting is legible in accordance with the following.

AC Voltage Range	Legible P.C.B. Setting	Fuse
90-110V	100	1.5A
105-129V	120	1.5A
198-242V	220	.8A
217-258V	240	.8A

***Note:** Chromatographic Peaks with a base width of 600 ms or less may be distorted by conventional strip chart recorders.

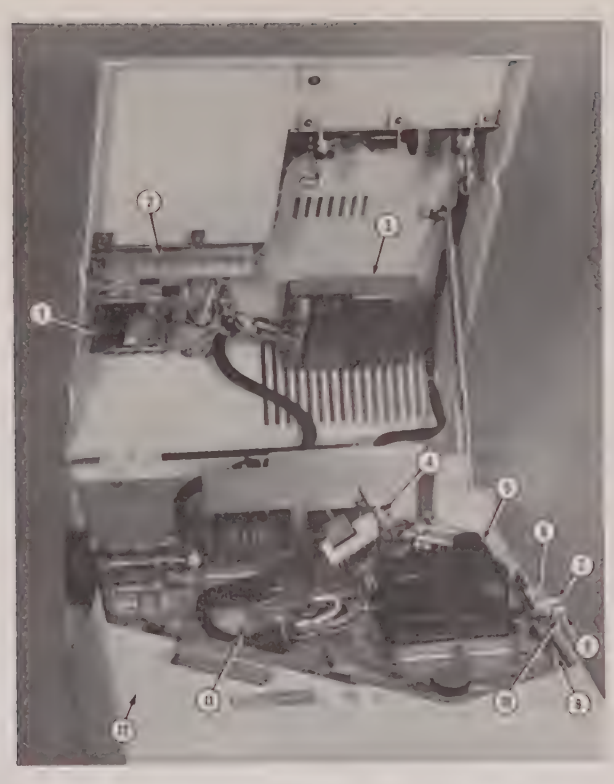


Figure 2-4 - LC-85B Shown with Cover Lifted

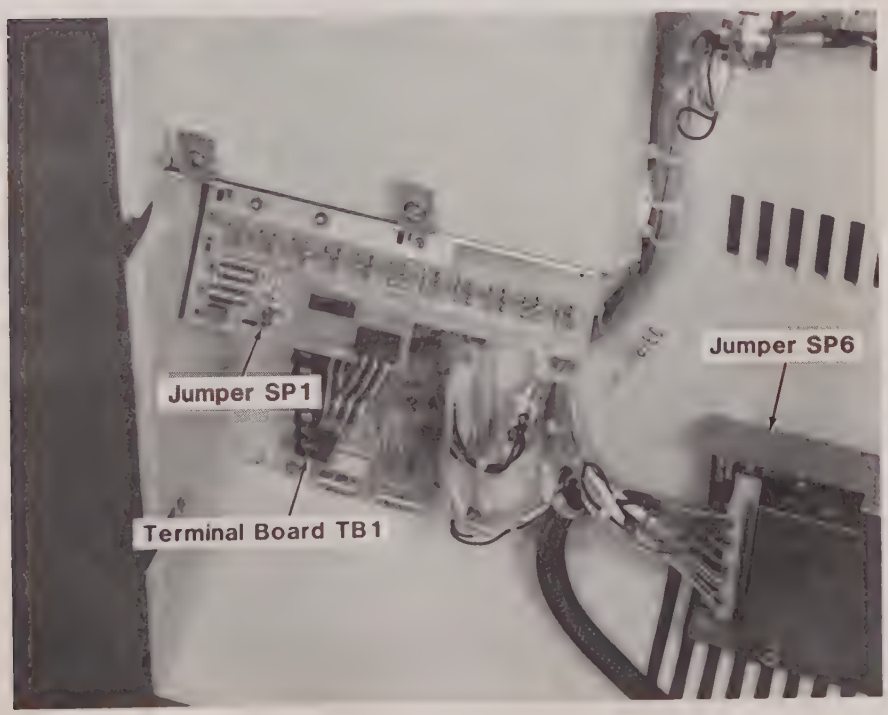


Figure 2-5 - Jumper and Terminal Locations

Important: It is not possible to change the instrument from one operating line voltage and frequency to another merely by changing the fuse and the orientation of the PCB. The filament transformer must also be changed if the operating frequency is to be changed. This should be performed only by a Perkin-Elmer Service Engineer.

Make certain that the proper fuse is inserted. Use either a 1.5A (P-E Part No. 0998-1624) or a 0.8A (P-E Part No 0998-1762) fuse depending on the line voltage. Note that both fuses are provided in the Spares Kit. Use only the proper Slo-Blo fuse. Push the printed circuit board back into the slot and rotate the FUSE PULL back into the normal position.

With the cover down and secured in place and the POWER switch OFF, connect the line cord to a properly-grounded AC outlet.

Important: All components of the system, e.g., LC-85B, the recorder, the solvent delivery module, etc., must share a common ground in the electrical supply system.

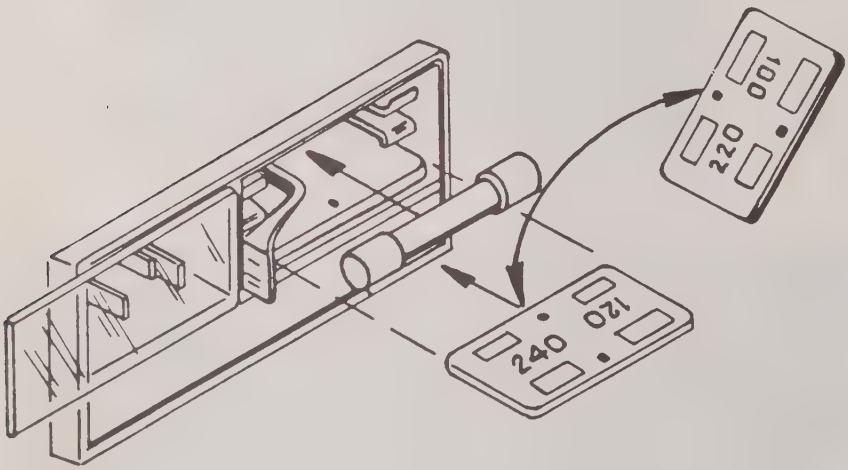


Figure 2-6 - Voltage Selection

2.5 INSTALLING THE DEUTERIUM LAMP

Install the lamp as follows:

1) Shut OFF the POWER.
2) Unplug the AC line cord.
3) Remove the detector cover.
4) Remove the guard from the lamp terminal strip. (See No. 3 in Figure 2-10.)

5) Press down on the front end of the lamp lever to release it. Swing the lever to the back of the LC-85B so that it frees the lamp bracket (see Figure 2-7).

6) Lift the magnetic lamp bracket from the detector. Do not lose the ozone seal gasket. (See Figure 2-8)

7) Install the lamp, (P-E Part No. 0271-1340).

8) DO NOT TOUCH the lamp window itself. Making certain that the seal gasket (P-E Part No. 0271-1277) is in place around the lamp window and place the prepared lamp in the lamp holder. Hold the lamp in position and place the magnet bracket and magnet assembly over the lamp as shown in Figure 2-7. The end stop on the bracket should rest against the bottom of the source.

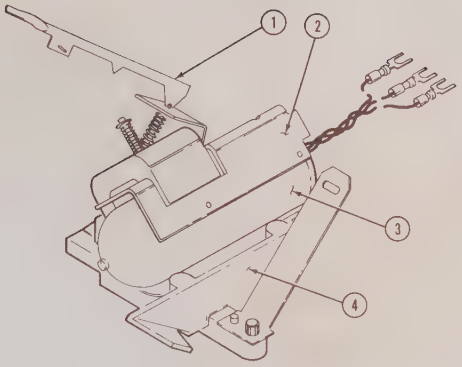
9) Using the center notch on the lamp lever to further locate the bracket as shown in Figure 2-8, press down on the lever to secure the pin on the lamp lever in the hold on the lever positioning clip.

10) Note the colors on the deuterium lamp terminal strip screws and connect the lamp wires to the deuterium lamp terminal strip in the following manner.

Wire Color	Terminal Position
BLU	3
RED	4
BLU	5

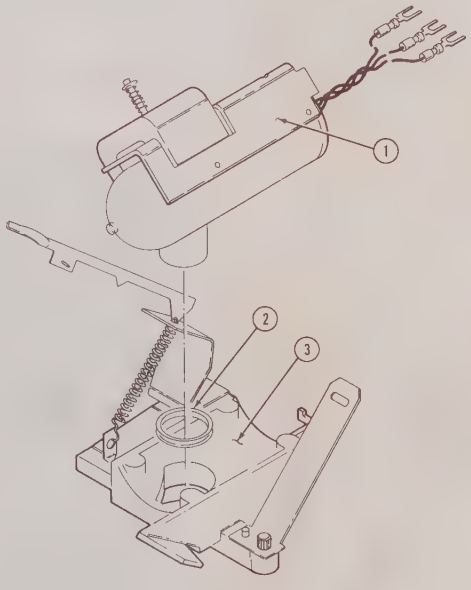
11) Replace the terminal strip guard and the terminal strip cover.

12) Align the deuterium lamp as described in Section 2.7.



1 Lever
2 Magnet and Lamp Bracket Assembly
3 Deuterium Source
4 Lamp Holder

Fig. 2-7 - Releasing the Lamp Lever



1 Lamp and Magnet Bracket
2 Ozone Seal Gasekt, 271-1277
3 Lamp Holder

Fig. 2-8 - Removing the Lamp

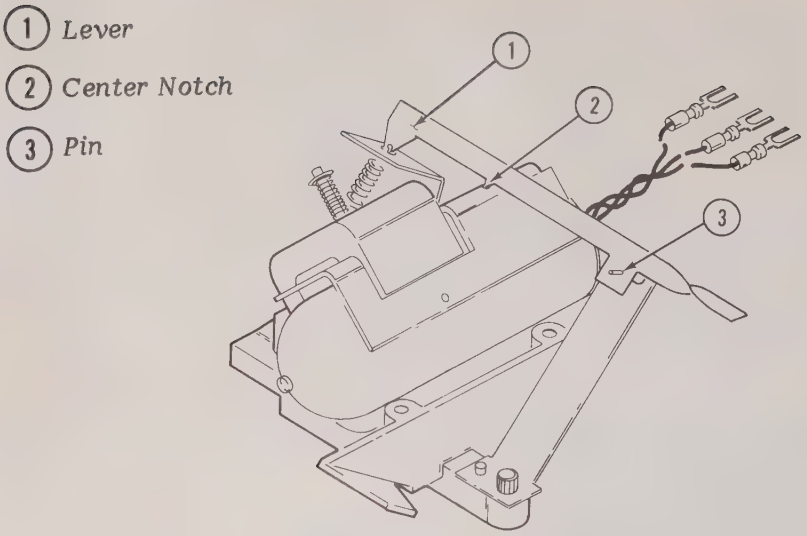


Figure 2-9 - Securing the Lamp

2.6 CHANGING THE FLOWCELL

It is possible to change the LC-85B from one cell to the other if required. Instructions are given in Section 4 for installing and aligning both cells. It is strongly urged, however, that once installed and aligned, the 1.4 µl cell be used exclusively. The narrow bore, thin walled tubing is very fragile and can be easily damaged.

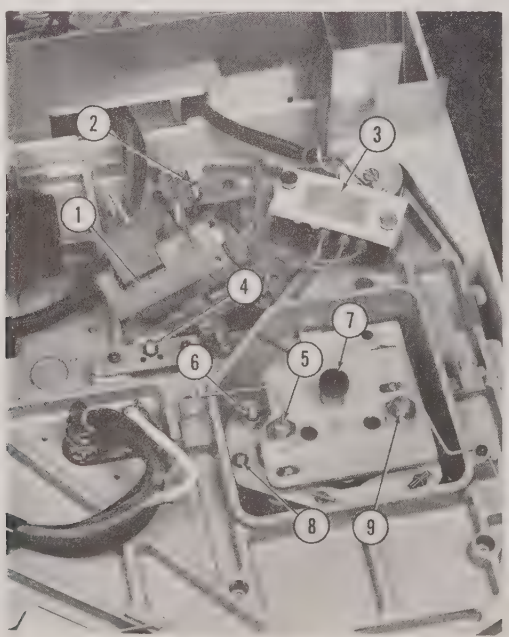


Figure 2-10 - Installation of the 8 µl Flowcell

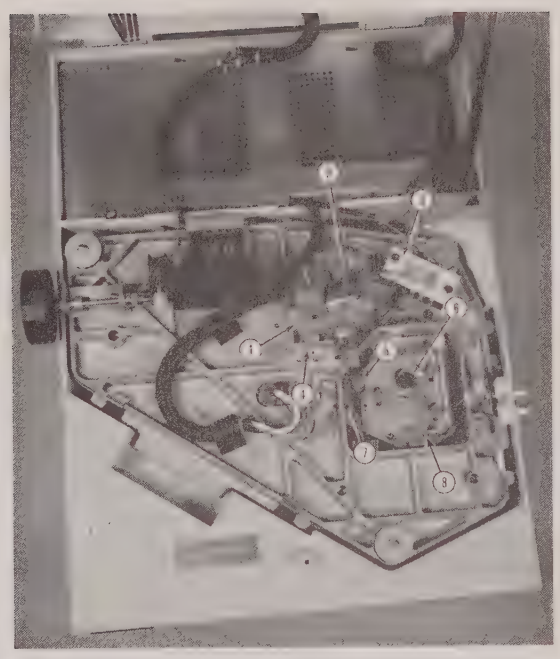


Figure 2-11 - Installation of the 1.4 µl Flowcell

Figure 2-12 - Cell Adjust Cover Installed

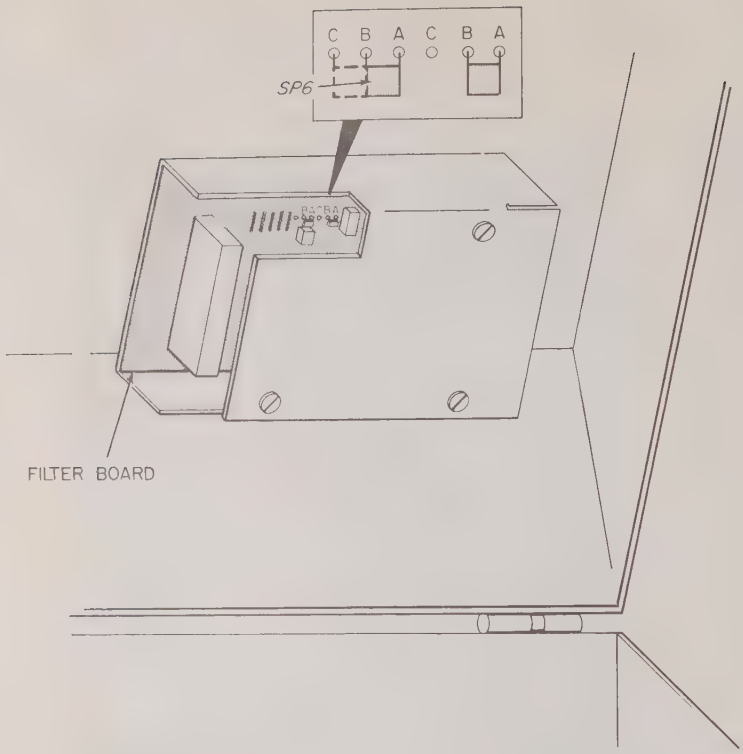


Figure 2-13 - Jumper SP6 and the Filter Board

2.7 ALIGNING THE DEUTERIUM LAMP

The LC-85B detector is a dual beam instrument which uses two separate photo-tubes and a dual electrometer which contains separate electornic circuitry for the sample and reference channels. Both the sample and reference circuit outputs are compared and differentiated to produce an output which represents a sample component being absorbed in the flow cell.

The D_2 lamp alignment is done by placing a fixed voltage on the sample electronics input and using the reference electronics to monitor the lamp intensity signal while adjusting the lamp. The output of the final differential amplifier will represent maximum lamp energy through the reference electronics which actually represents maximum absorbance.

When adjusting the flow cell, the fixed voltage is removed from the sample electronics input and the flow cell is adjusted so that maximum UV light strikes the sample photo-tube when there is no sample present in the flow cell. Since the lamp is not being adjusted the reference channel now has a fixed voltage on its input, provided by the UV lamp energy striking the reference photo-tube.

While adjusting the flow cell, the output of the differential amplifier on the electrometer assembly represents maximum energy striking the sample photo-tube with no sample in the flowcell which is minimum absorbance. It is important to understand that because the sample circuit on the electrometer feeds the opposite input of the final stage differential amplifier than the reference circuit, the flowcell peak will show up on the chart recorder in the opposite direction from the peaking of the lamp.

Warning: Alignment procedures in Sections 2.7, 2.8 and 2.9 are performed with the detector cover raised and power on. Although high voltage terminal boards and terminal points are insulated, caution must always be exercised near live circuits.

Never look directly at the deuterium lamp when it is on. To avoid UV damage to the eyes always wear ultraviolet opaque safety glasses whenever the cover is lifted and the lamp is on. The lamp is hot. Allow the lamp to cool completely or wear protective gloves to avoid burns when handling the lamp.

1) Turn the POWER switch ON. Make certain that the solvent flow through the cell is stopped and determine that it is transparent at a wavelength of 240 nm. If in doubt, blow the cell dry with a stream of dry nitrogen.

2) Allow the lamp to warm up for at least 10 minutes.

3) Set the WAVELENGTH control at 240 nm. Set the ABSORBANCE$_{1cm}$ selector at 128 (1.28A$_{1cm}$). Short the recorder input (some recorders have a "zero" switch for this purpose). With the signal input to the recorder shorted, adjust the recorder zero control to bring the pen to mid-scale on the chart. Open the recorder input signal short and use the LC-85B ZERO control to bring the pen back to mid-scale.

4) Lift the detector cover.

5) Remove the flowcell cover and replace it with the cell adjust cover (0271-1298) supplied (See Figure 2-12).

6) Turn the cell horizontal rotation adjustment screw (item 7 in Figure 2-11) counterclockwise exactly 5 complete rotations.

7) Using insulated needle nose pliers carefully lift jumper SP6, located on the filter board (see Figure 2-13) from the AB position and move it one pin to the left to the BC position.

8) Use the LC-85B ZERO control to bring the pen on scale.

9) Adjust the lamp linear adjustment (item 2, Figure 4-2) and the lamp tilt adjustment (item 4, Figure 4-2) screws alternately until a maximum positive voltage is obtained. This is a maximum deflection in the absorbance peak direction.

10) Place jumper SP6 back into the AB position (see Figure 2-13). The source is then aligned.

2.8 ALIGNING THE 8 µl FLOWCELL

1) Rotate the horizontal adjustment screw exactly 5 complete rotations in the clockwise direction. (Note: This is a reversal of the rotation of step (6) in the procedure (2.7) for adjusting the Deuterium Lamp.)

2) Carefully rotate the cell horizontal rotation (item 7, Figure 4-2) and the cell vertical tilt (item 5, Figure 4-2) adjustment screws alternately until a minimum absorbance indication is obtained. This is a maximum deflection in the baseline direction. It may go to a negative voltage. Usually less than a single turn is sufficient to bring the cell into alignment. Avoid making drastic changes in both directions which would remove the cell completely from the optical path.

3) Replace the cell adjust cell cover with the operation cover removed in step 5 of the Lamp Adjustment procedure (2.7).

2.9 ALIGNING THE 1.4 ul FLOWCELL

Because the 1.4 µl cell utilizes a lens as a window this flowcell has an additional adjustment for optimizing the focus of the exit slit.

1) Rotate the horizontal adjustment screw exactly 5 complete rotations in the clockwise direction. (Note: This is a reversal of the rotation of step (6) in the procedure for adjusting the Deuterium Lamp.)

2) Rotate the cell horizontal adjustment (item 7, Figure 4-2) and the cell vertical tilt (item 5, Figure 4-2) adjustment screws alternately until a minimum absorbance indication is obtained. This is a maximum deflection in the baseline direction. It may go to a negative voltage.

3) Adjust the focus travel screw (item 8, Figure 4-2) for a maximum voltage in the negative direction.

4) Replace the cell adjust cover with the operation cover removed in step (5) of the Lamp Adjustment procedure (2.7).

> **Note**: If, upon installation, a satisfactory baseline cannot be obtained, an Optical Compartment purge may be necessary. See the Service Section of this manual.

SECTION 3
OPERATING INSTRUCTIONS

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3 OPERATING INSTRUCTIONS

See the end of this Section for a summary of these instructions.

3.1 Controls and Indicators

The item numbers below correspond to item numbers in Figure 3-1.

1. ABSORBANCE$_{1cm}$ Range Selector - Eleven-positon, push-button binary attenuator adjusts the detector signal to the recorder. One of the attenuator buttons must be depressed for the detector to operate. The attenuator buttons provide a range of 0.005 to 5.12 Absorbance$_{1cm}$.

2. RESPONSE Switch - 3-position switch which allows selection of the electronic response time of the LC-85B to satisfy the needs of the particular analysis. The positions available are 20 ms, 200 ms and 2000 ms.

3. POWER - Placing the power switch in the ON (up) position provides power to the LC-85B. The POWER pilot light (in the ON-OFF switch) illuminates to verify that the power is on.

4. ZERO Control - Turning this control sets the recorder baseline and data output.

5. Screw Cover Cap - The screw under this cap secures the detector cover and must be in place for normal operation.

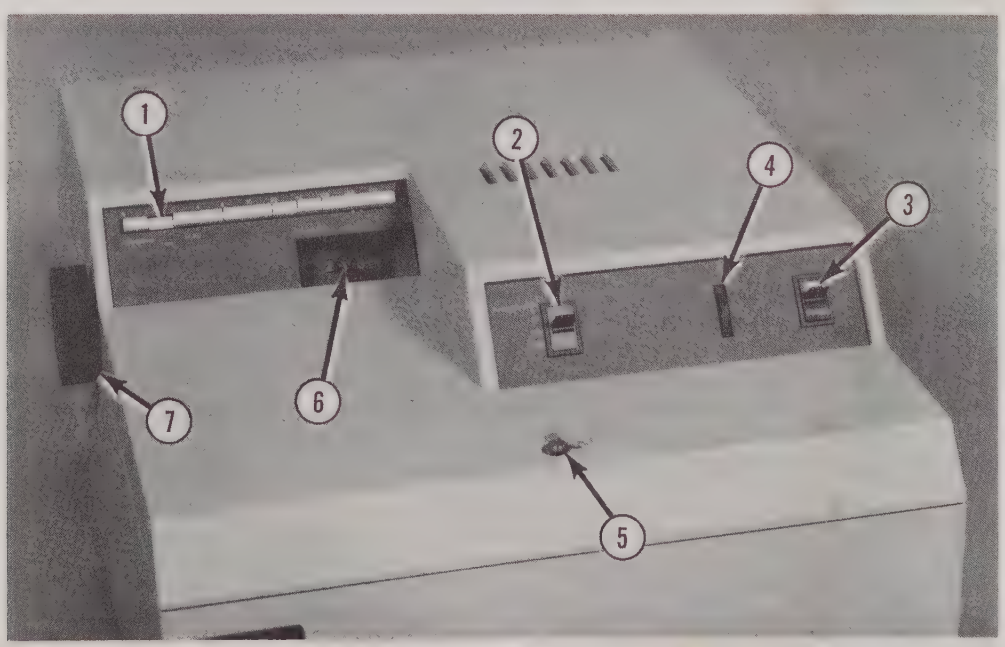

Figure 3-1 - LC-85B Controls and Indicators

6. WAVELENGTH (nm) Indicator - This wheel indicator provides a three digit and first decimal place readout of the wavelength setting, graduated in 0.2 nm increments, readable to 0.1 nm.

7. Wavelength Control Knob - Turning this knob sets the detector wavelength.

   Important: 1) Do not force the control knob below the 000.0 nm setting or above the 900.0 nm setting. 2) For repeatability, always approach the desired wavelength from the low side, i.e., set 250 nm by starting at 200 nm. The spectral region of the instrument is 190-600 nm.

3.2 DETECTOR OPERATION

3.2.1 General Instructions

Important: Do not place anything on the top surface of the detector. Also do not permit any solvent to leak into the detector interior.

1) Connect the system as required for the analysis and set up the detector as described in the Installation Section.

2) Place the POWER switch in the ON (up) position. Allow approximately 30 minutes for the deuterium lamp to warm up.

3) Set the wavelength control to the appropriate valve. Never turn the control to a setting below 000.0 or above 900.0. The useful spectral region of the instrument is 190-600 nm. Always approach the desired setting from the low side to ensure repeatability.

4) Use the ZERO control to set the recorder baseline.

5) The ABSORBANCE setting required will depend on the concentration of the components. Attenuate the signal as necessary to keep the peaks on scale. The LC-85B is set for use with a 10 mV full scale recorder.

6) Permit the system to equilibrate by running sufficient solvent through the column and detector until pure solvent flow is established. A flat starting baseline on the recorder is essential for a repeatable analyses.

7) Select the appropriate response time position to best separate the chromatographic peaks from any detector noise. A response time too much shorter than the duration of a peak passes more detector noise than necessary. A response time which is longer than the duration of a peak will reduce resolution, causing a broadening of the peak and a reduction in its height. To minimize loss in resolution the detector response time should be less than one-third of the base width of the chromatographic peak. Therefore, select the appropriate response as follows:

   Detector Response

   20 ms for high speed LC peaks 60 ms to 600 ms wide.
   200 ms for conventional LC peaks 0.6 secs. to 6 secs. wide.
   2000 ms for high noise situations, peak widths greater than 6 seconds.

8) Inject the sample.

3.2.2 Use of the ABSORBANCE$_{1cm}$ Range Selector

If the largest peak is less than 50% of full scale deflection on the recorder, choose a lower setting. If any of the peaks is too large, cut the detector response by choosing a higher setting. The settings available are: 0.5,1,2,4,8,16,32,64,128,256,512. Changing the recorder signal by changing the range does not affect the data out (1V, 10V) signal.

3.2.3 Zero Procedure

A single zero control is provided to adjust the recorder pen to baseline. The zero control affects both the recorder and data outputs.

1) Use the ZERO control to bring the recorder baseline to zero.

2) Repeat the zero procedure when the wavelength is changed.

3) If you are unable to zero the detector, perform the checks provided in the Service Section.

3.3 FLOW CELL SELECTION

The LC-85B is shipped with either the 8 µl flow cell (P-E Part No. 0271-0338) or the low-volume 1.4 microliter high-speed cell (P-E Part No. 0271-0232).

If a detector with a 8 µl cell is ordered, and the cell is replaced with a 1.4 µl cell, a low volume injector and connecting tubing must be used. For instructions for installing the cell kit, see the Service Section. It is strongly urged, however, that once installed and aligned, the 1.4 µl cell be used exclusively. The narrow bore, thin walled tubing is very fragile and can be easily damaged.

> Important: Use the proper cell for the task being performed. Use only the standard 8 µl flow cell when doing prep scale separations at flow rates above 10 milliliters per minute. At very high flow rates, the pressure limit of 1000 psi (7 MPa) of the high speed 1.4 µl cell can be exceeded, causing the cell to leak and resulting in damage to the detector optics.
>
> When working with short, high-speed columns, or other high resolution columns a 1.4 µl cell must be used to prevent loss of resolution of the separated peaks. If an LC-85B detector is ordered with a 1.4 µl cell installed (P-E Part Nos. 0271-0033 and 0271-0034), the low volume injector and low volume tubing must be used.

3.4 SHUTDOWN PROCEDURES

3.4.1 Short-Term Shutdown (Overnight and Weekends)

Observe all the precautions outlined in Section 1.3, Warnings and Precautions with respect to hazardous solvents and/or those which may form harmful deposits or by-products.

1) Remove buffer salts from the flowcell and flush with water as evaporation will leave salt crystals which may form harmful deposits.

   Any damage caused by precipitating buffer salts in capillary tubing or any damage

resulting from this condition are specifically excluded from Warranty. The tubing used in the 1.4 µl flow cell is only 127 microns (0.005") (0.13 mm) in diameter and is easily plugged.

2) Chloroform or solvents which can decompose to form hydrochloric acid should not be left in the system overnight.

Note: Solvents, including water, exposed to the environment for more than 24 hours should be refiltered before use or discarded.

3) Shut off the power to the pumps and the Model LC-85B Detector.

Important: The detector deuterium source has a finite life. Turn if off when not in use for an extended period of time.

4) If a data acquisition device is in use, consult the instructions for that device. (Turning off the power to the data system may not be advantageous if Methods and Data files have not been permanently stored.)

3.4.2 Long-Term Shutdown

1) Shut off the power to the detector.

2) Remove from the flowcell any solvent, such as carbon tetrachloride or tetrohydrofurane, which might form hazardous by-products.

3) Remove buffer salts from the flowcell and flush with water as evaporation will leave salt crystals which may form harmful deposits.

Any damage caused by precipitating buffer salts in capillary tubing or any damage resulting from this condition are sepcifically excluded from Warranty. The tubing used in the 1.4 µl flow cell is only 127 microns (0.005") (0.13 mm) in diameter and is easily plugged.

4) For long term storage it is recommended that the cell be finally flushed with isopropanol and then blown dry with dry nitrogen.

3.5 OPERATING SUMMARY

1) Connect the detector INlet line to the column outlet. If unions are used, make certain that they are of the zero-dead-volume-type (P-E Part No. 0990-3259).

2) Connect the detector signal outputs to the recorder and/or data system. (See the Installation Section.)

3) Turn on the LC-85B power. Allow approximately 30 minutes minimum warm-up time. (Allow approximately 1 hour for maximum sensitivity setting.)

4) Select appropriate solvent composition and flow rate. Do not exceed 1000 psi cell pressure for the 1.4 µl cell; 4000 psi for the 8 µl cell. Begin pumping. Make certain that all plumbing connections are leak-free and that the pressure is stable.

Cell pressure is measured directly by removing the column and measuring directly all backpressure at the desired flow rate. Cell pressure is measured indirectly by noting the backpressure with the detector removed and then replaced in the system and subtracted.

5) Allow the flow rate to stabilize.

6) Use the detector WAVELENGTH dial to select a wavelength appropriate for the analysis. Make certain that the wavelength chosen is above the UV cutoff for the solvent(s) in use. (If the sample absorbance wavelength is unknown, use 250 nm initially as many compounds absorb at this wavelength.)

7) Select the $ABSORBANCE_{1cm}$ setting. (The $ABSORBANCE_{1cm}$ push button control is used to keep the peaks on-scale. A setting of 16 x 0.01 is typical.)

8) Observe the baseline until it is stable. Adjust the signal to 0 to 10% of the recorder scale with the ZERO control.

9) Inject the sample.

   a) If the peaks go off-scale, select a higher number $ABSORBANCE_{1cm}$ setting to make the peaks smaller. (If a data acquisition device is in use, peaks may be allowed to go off-scale.)

   b) If the peaks are too small, use a lower number $ABSORBANCE_{1cm}$ setting.

SECTION 4
SERVICE AND MAINTENANCE

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4 SERVICE AND MAINTENANCE

4.1 GENERAL PRECAUTIONS

1) Switch the POWER Off and unplug the AC line cord whenever it is necessary to perform maintenance on the LC-85B detector such as changing the flowcell, deuterium lamp or the line fuse. Certain procedures such as aligning the deuterium lamp and the flowcell require that the detector cover be raised while power is on. Although high voltage terminal boards and terminal points are insulated, caution must always be exercised near live circuits.

2) Never look directly at the deuterium lamp when it is on. To avoid UV damage to the eyes, always wear ultraviolet opaque safety glasses whenever the cover is lifted and the lamp is on. The lamp is hot. Allow the lamp to cool completely or wear protective gloves to avoid burns when handling the lamp.

4.2 TROUBLESHOOTING

When detector response and performance changes suddenly for no apparent reason, the following sequence may be used to narrow down the number of possible causes.

1) Make certain that power is supplied to LC-85B. Make certain that the POWER switch is in the ON position, and that the AC line cord is plugged into a working outlet and that the fuses are proper and in good condition.

2) Turn the power OFF and unplug the AC line cord. Verify that the deuterium lamp wires are securely connected (refer to Section 4.4 (10))

3) Make certain that the deuterium lamp is aligned properly (see Section 4.5).

4) Verify that the cell is aligned (see Sections 4-8 and 4-9).

5) Consult the Troubleshooting Chart given in Table 4-1.

TABLE 4-1 LC-85B TROUBLESHOOTING CHART

OBSERVATION	PROBABLE CAUSE	REMEDY
No power to detector.	1. Fuse F101 is blown.	1. Replace the fuse with an appropriate one (see Section 4.3).
	2. High voltage fuse F102 is blown.	2. Replace the fuse with an appropriate one (see Section 4.3.
	3. Low voltage power supply board is faulty.	3. Call a Perkin-Elmer Service Engineer.
Deuterium lamp does not light.	1. Deuterium lamp wires are loose.	1. Turn OFF the POWER, unplug the LC-85B and reconnect the wires (Figure 4-2).
	2. Deuterium lamp is faulty.	2. Replace the lamp and align it (Section 4.4 and 4.5).
	3. Lamp driver board is faulty.	3. Call a Perkin-Elmer Service Engineer to replace the lamp driver board.
No detector response.	1. Deuterium lamp is out of position.	1. Align the deuterium lamp (Section 4.5).
	2. Foreign object in the sample compartment.	2. Clean the sample cell (Section 4.6).
	3. Cell assembly is out of position.	3. Realign the cell assembly (Section 4.7).
	4. Wavelength setting is below cutoff wavelength of the solvent.	4. Change to appropriate wavelength.
Low response and 0A cannot be set.	1. Lamp mirror is slightly out of position.	1. Align the lamp (Section 4.5).
	2. Foreign object in the sample compartment.	2. Clean the sample cell (Section 4.6).
	3. Deuterium lamp is badly darkened.	3. Replace the lamp and align the new one. (Sections 4.4 and 4.5).
	4. Sample compartment quartz windows are dirty.	4. Clean the quartz window (Section 4.6).
	5. Signal amplifiers may require service.	5. Call a Perkin-Elmer Service Engineer.
Detector response is unsteady regardless of the position of the controls.		
1. Deuterium lamp flickers.	1a. Deuterium lamp wire connections are loose.	1a. Reinstall the lamp and realign. (Sections 4.4 and 4.5).
	1b. Deuterium lamp is faulty.	1b. Replace the lamp and align the new one.
2. Dueterium lamp is properly lit and aligned properly.	2a. Solvent is interferring with detector response.	2a. Remove source of solvent interference (Precautions, Section 1)
	2b. Cell holder is loose.	2b. Tighten cell holder.
	2c. Excessive line voltage fluctuation.	2c. Check line voltage.
	2d. Sample is unstable.	2d. Examine sample for change due to degradation, fading, settling of precipitate, etc. Clean the cell.
Indications are incorrect		
1. Successive indications on the same material are not reproducible.	1. False chemical standard.	1. Check sample for change due to degradation, fading, settling of precipitate, etc.
2. Indications do not agree with previous determinations.	2a. Change in chemicals or technique from previous runs.	2a. Use the same technique.
	2b. Laboratory environment contains UV-absorbing vapors.	2b. Purge the cell with clean, dry nitrogen. If conditions persist, employ a slight nitrogen purge of the monochromator. (See Section 2, Installation.)
3. Calibration is off.	3. Wavelength calibration is off.	3. Check wavelength calibration (Seciton 4.10).

4.3 FUSE REPLACEMENT

4.3.1 Line Fuse F101

To replace fuse F101 on the AC input plate, see Figure 4-1 and do the following:

1) Turn the POWER switch OFF.

2) Unplug the AC line cord.

3) Slide the clear plastic fuse cover on the left side of the LC-85B all the way to the left. Rotate fuse, pull to left and examine fuse.

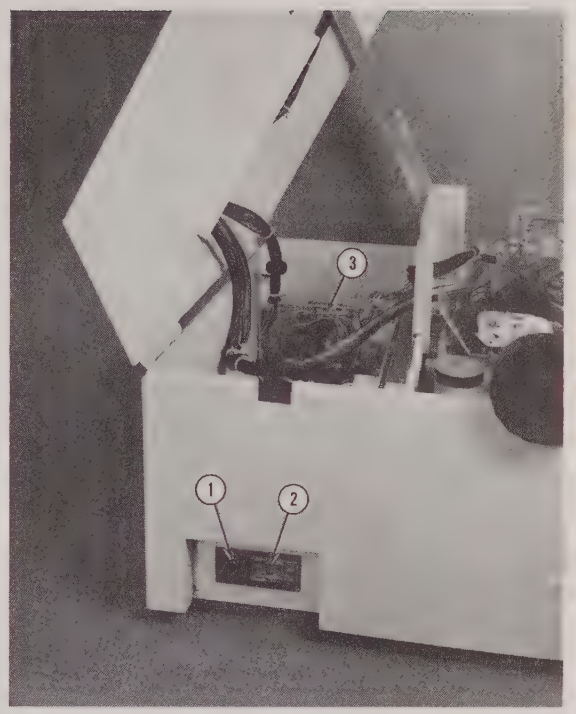
4) If the fuse is blown, remove it and discard it.

5) Place a new fuse in the fuse holder. Use either a 1.5A fuse (P-E Part No. 0998-1624) for a 117V unit or a 0.8A fuse (P-E Part No. 0998-1762) for a 234V unit. Use only slo-blo fuses.

6) Rotate fuse pull back to its normal positon and and slide the plastic cover back to the right.

7) Plug in the AC line cord.

8) Turn the POWER On.



1 Line Cord Connector
2 F101 Line Fuse
3 F102 HV Fuse (beneath cover)

Figure 4-1 - Fuse and Line Cord Connector Location

4.3.2 Deuterium Lamp High Voltage Fuse F102

1) Turn the power switch off.

2) Unplug the AC line cord.

3) See Figure 4-1. With the instrument cover lifted, locate the high voltage fuse beneath the cover as shown.

4) Remove the fuse from the fuse holder (remove the protective shield if necessary) and replace it with a 0.5A fuse (P-E Part No. 0998-1622). Replace the cover and restore power.

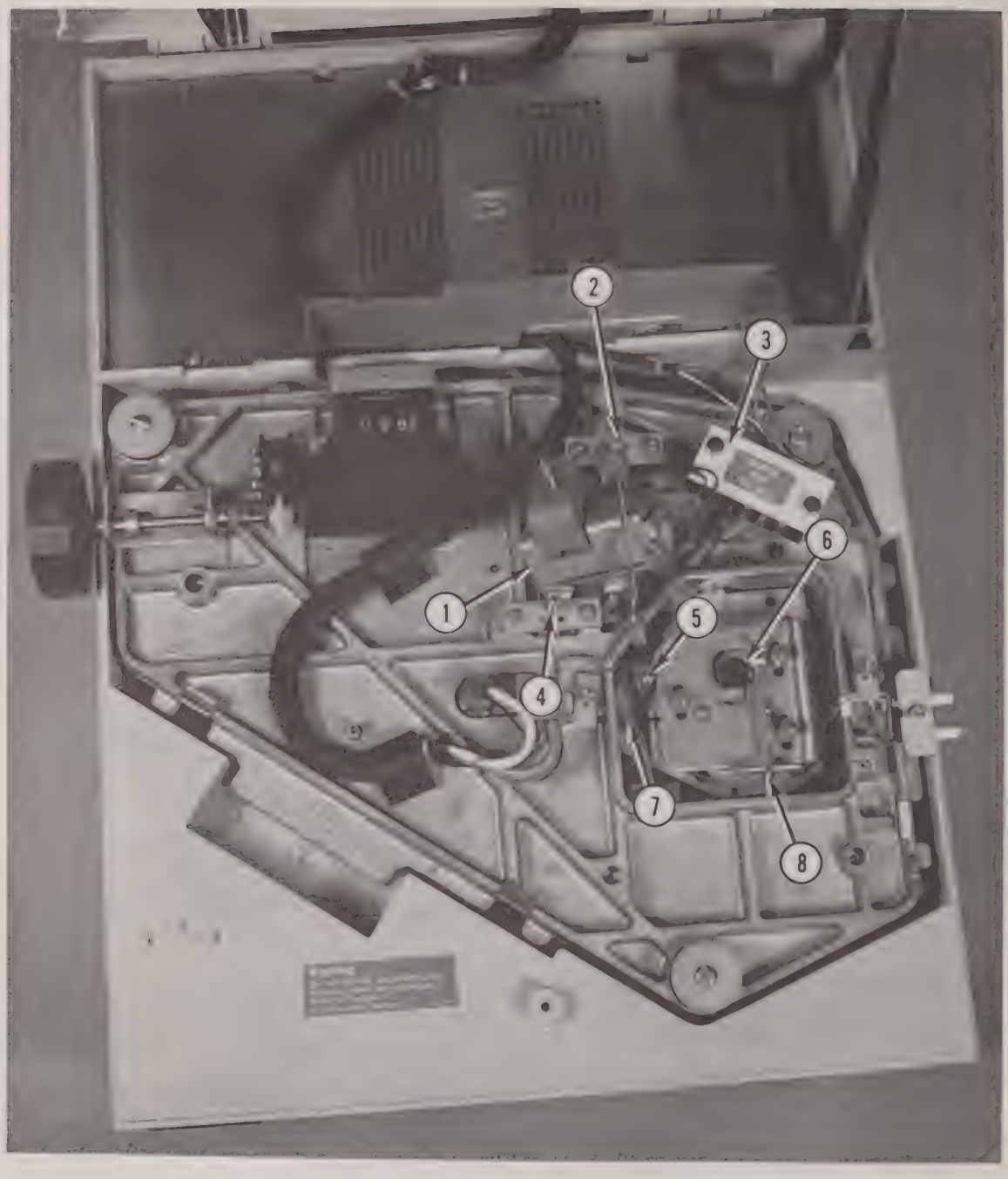


Figure 4-2 - Detector Interior

4.4 REPLACING THE DEUTERIUM LAMP

Replace the lamp as follows:

1) Shut OFF the POWER. Unplug the AC line cord. Lift the detector cover.

2) Remove the guard from the lamp terminal strip (see item 3, Figure 4-2).

3) Disconnect the three lamp leads.

Warning: Wear thermal protective gloves to protect your hand from burns or wait for the lamp to cool.

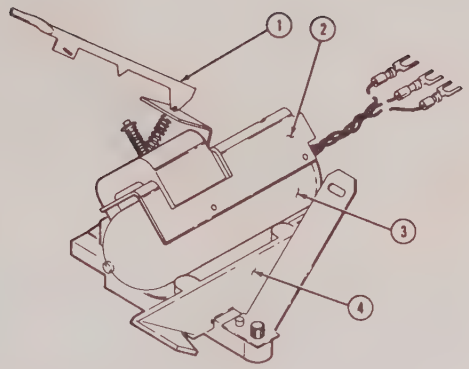
4) Press down on the front end of the lamp lever to release it. Switch the lever to the back of the LC-85B so that it frees the lamp and lamp bracket. (See Figure 4-3.)

5) Lift the lamp and the magnet lamp bracket from the detector. Do not lose the ozone seal gasket. (See Figure 4-4.)

6) Separate the magnet bracket (with the magnet attached) from the lamp.

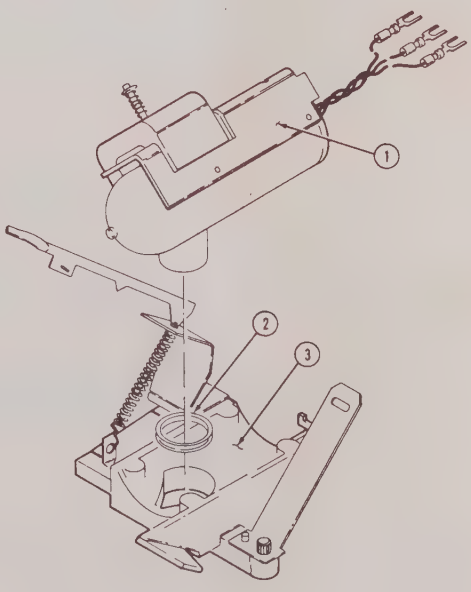
7) Discard the old lamp. Replace the lamp with a new deuterium source (P-E Part No. 0271-1340).

8) DO NOT TOUCH the lamp window itself. Making certain that the seal gasket (P-E Part No. 0271-1277) is in place around the lamp window and place the prepared lamp in the lamp holder. Hold the lamp in position and place the magnet bracket and magnet assembly over the lamp as shown in Figure 4-3. The end stop on the bracket should rest against the bottom of the source.



1 Lever
2 Magnet and Lamp Bracket Assembly
3 Deuterium Source
4 Lamp Holder

Figure 4-3 - Releasing the Lamp Lever



1 Lamp and Magnet Bracket
2 Ozone Seal Gasket, 271-1277
3 Lamp Holder

Figure 4-4 - Removing the Lamp

9) Using the center notch on the lamp lever to further locate the bracket as shown in Figure 4-5, press down on the lever to secure the pin on the lamp lever in the hole on the lever positioning clip.

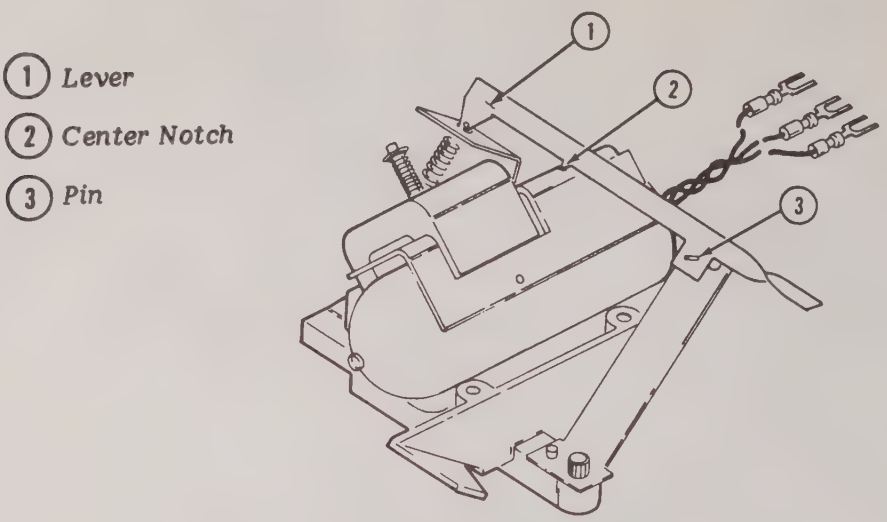


Figure 4-5 - Securing the Lamp

10) Note the colors on the deuterium lamp terminal strip screws and connect the lamp wires to the deuterium lamp terminal strip in the following manner.

Wire Color	Terminal Position
BLU	3
RED	4
BLU	5

11) Replace the terminal strip guard and the terminal strip cover.

12) Align the deuterium lamp as described in Section 4.5.

4.5 ALIGNING THE DEUTERIUM LAMP

> **Warning**: Never look directly at the deuterium lamp when it is on. To avoid UV damage to the eyes always wear ultraviolet opaque safety glasses whenever the cover is lifted and the lamp is on. The lamp is hot. Allow the lamp to cool completely or wear protective gloves to avoid burns when handling the lamp.

Align the deuterium lamp whenever the lamp has been disturbed or replaced. Make certain that the recorder is connected and operating. This procedure should be performed in a dimly-lit room.

The LC-85B detector is a dual beam instrument which uses two separate photo-tubes and a dual electrometer which contains separate electronic circuitry for the sample and reference channels. Both the sample and reference circuit outputs are compared and differentiated to produce an output which represents a sample component being absorbed in the flow cell.

The D_2 lamp alignment is done by placing a fixed voltage on the sample electronics input and using the reference electronics to monitor the lamp intensity signal while adjusting the lamp. The output of the final differential amplifier will represent maximum lamp energy through the reference electroncis which actually represents maximum absorbance.

When adjusting the flow cell, the fixed voltage is removed from the sample electronics input and the flow cell is adjusted so that maximum UV light strikes the sample photo-tube when there is no sample present in the flow cell. Since the lamp is not being adjusted the reference channel now has a fixed voltage on its input, provided by the UV lamp energy striking the reference photo-tube.

While adjusting the flow cell, the output of the differential amplifier on the electrometer assembly represents maximum energy striking the sample photo-tube with no sample in the flow cell which is minimum absorbance. It is important to understand that because the sample circuit on the electrometer feeds the opposite input of the final stage differential amplifier than the reference circuit, the flow cell peak will show up on the chart recorder in the opposite direction from the peaking of the lamp.

1) Turn the POWER switch ON. Make certain that the solvent flow through the cell is stopped. The solvent must be transparent to 240 nm. If in doubt, clean and dry the cell with a stream of dry nitrogen.

2) Set the WAVELENGTH control at 240 nm. Set the $ABSORBANCE_{1cm}$ selector at 128 ($1.28A_{1cm}$). Short the recorder input (some recorders have a "zero" switch for this purpose). With the signal input to the recorder shorted, adjust the recorder zero control to bring the pen to mid-scale on the chart. Open the recorder input signal short and use the LC-85B ZERO control to bring the pen back to mid-scale.

3) Wait 10 minutes to allow the lamp to warm up.

4) Lift the detector cover.

5) Remove the cell cover and replace it with the cell-adjust cover, supplied (see Figure 4-6).

6) Turn the cell horizontal rotation adjustment screw (item 7 in Figure 4-2) counterclockwise exactly 5 complete rotations.

7) Using insulated needle nose pliers carefully lift jumper SP6, located on the filter board (see Figure 4-12) from the AB position and move it one pin to the left to the BC position.

8) Use the detector ZERO control to bring the pen on scale.

9) Adjust the lamp linear adjustment (item 2, Figure 4-2) and the lamp tilt adjustment (item 4, Figure 4-2) screws alternately until a maximum positive voltage is obtained. This is a maximum deflection in the absorbance peak direction.

10) Place jumper SP6 back into the AB position (see Figure 4-12). The source is then aligned.

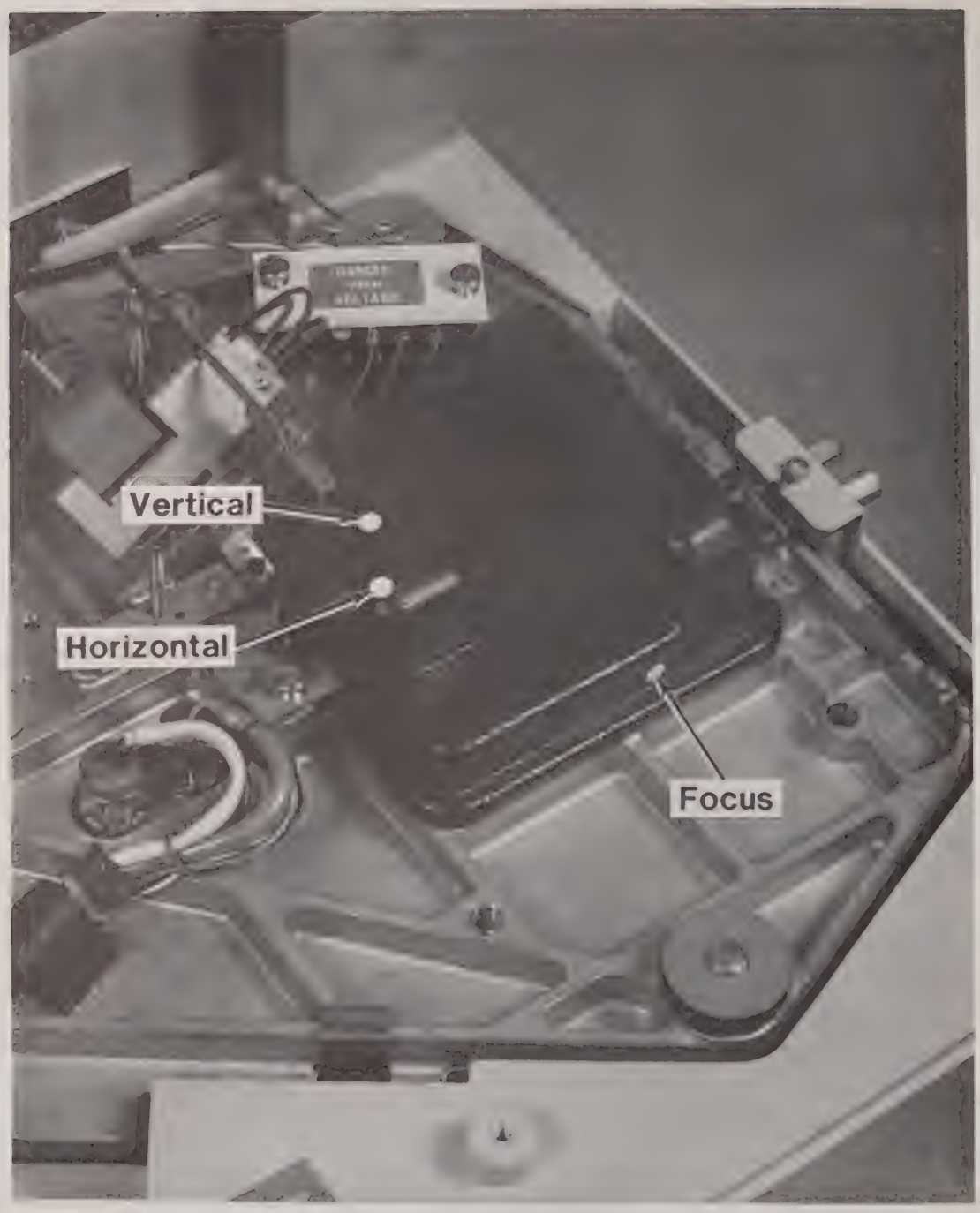
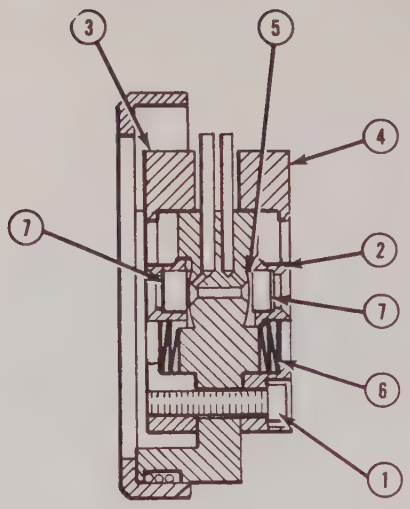


Figure 4-6 - Cell Adjust Cover



1. Screw, 4-40 x 1/4-in. Soc. Hd. Cap. St. Stl.
2. Holder, Cell Window
3. Clamp, Cell Window, L.H.
4. Clamp, Cell Window, R.H.
5. Seal, Cell Window
6. Spring, Disk
7. Clear Quartz Window

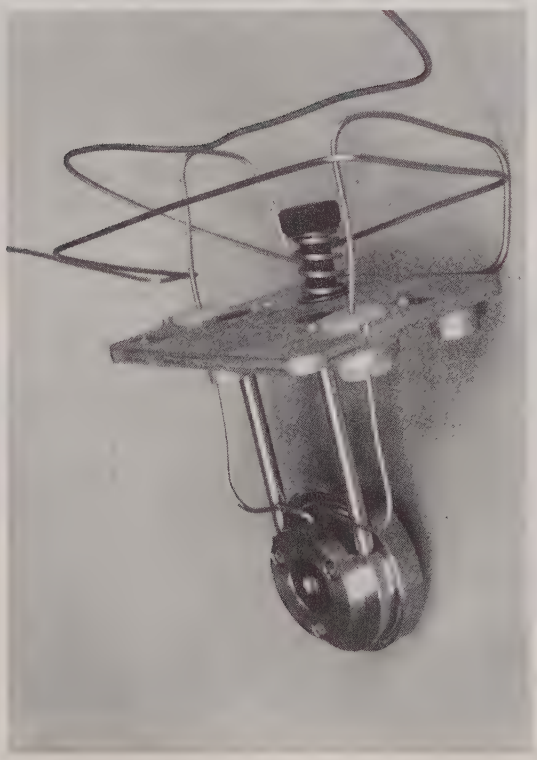


Figure 4-7 - The 8µl Flowcell
The 8µl Flowcell has 2 Windows.

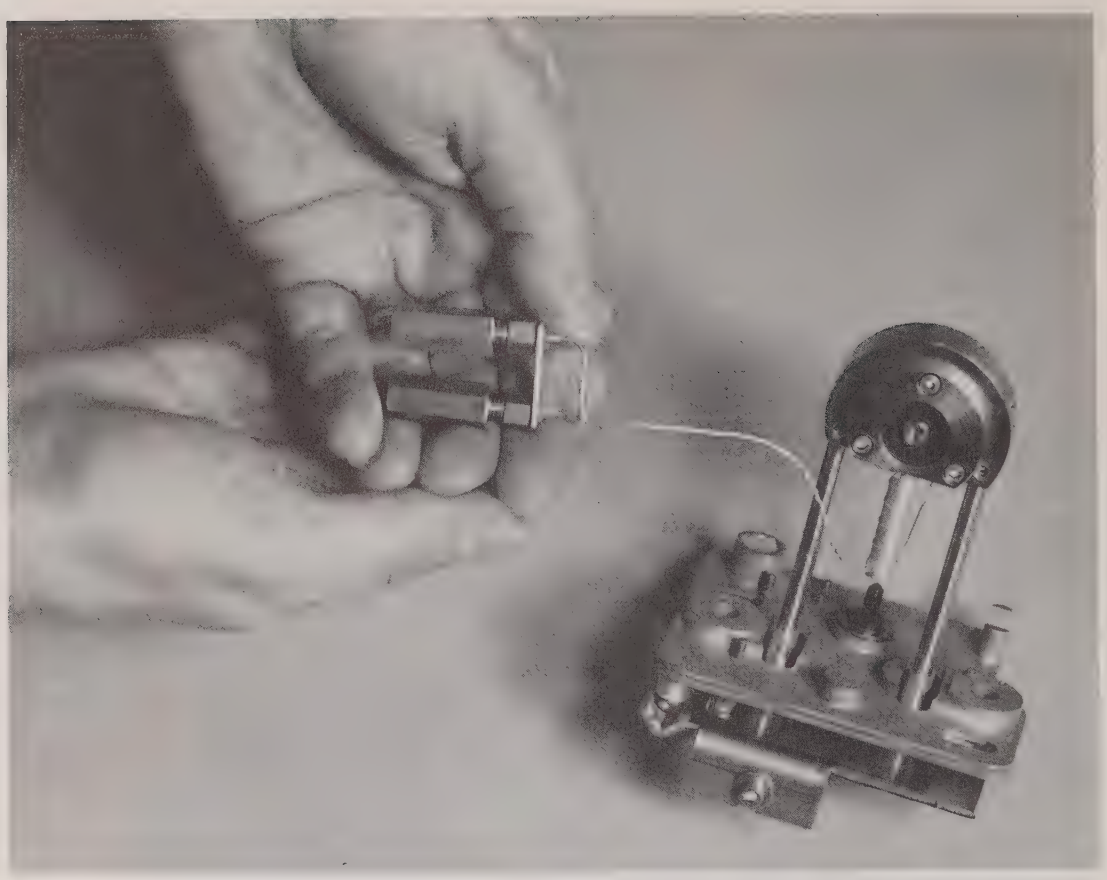


Figure 4-8 - The 1.4 µl Flowcell
The 1.4 µl Flowcell has 1 Window and 1 Focusing Lens.

4.6 REMOVING THE SAMPLING CELL FOR CLEANING

If it becomes impossible to zero the baseline with known clean solvents in the cell, or if baseline noise is experienced, the cell should be examined for cleanliness. If the windows appear to be clean, the problem may result from the presence of a "bubble" of insoluble liquid trapped in the cell (such as hexane in a water system) which can be flushed out with a mutually misicible solvent such as isopropanol. This can easily be determined by proceeding through step (6) below, then flushing the cell and replacing it as described in steps 15 and 16.

1) Turn the POWER Off.

2) Disconnect the AC line cord.

3) Be sure the pump (if connected) is stopped and the pressure in the flowcell relieved.

4) Remove the cap over the screw which secures the detector's cover, loosen the screw and lift the cover.

5) Remove the cell cover and loosen the black, knurled thumb screw holding the flowcell in its kinematic mount. On the 1.4 µl cell, disconnect the column and waste line from the flowcell's inlet and outlet couplings and then remove the clamp securing the couplings to the detector by loosening the #8-32 socket head screw shown in Figure 4-2. In removing the 1.4 µl cell from the detector the couplings must be supported to prevent damage to the fragile inlet and outlet lines. In subsequent steps move the cell slowly when changing its position, keeping in mind the mass of the couplings at the end of the tubes.

6) Lift the sampling cell straight out of the detector. Inspect the cell windows for obvious problems such as bubbles, cracks, or dirt. If further disassembly is required, proceed to step 7.

7) Lay the sampling cell down so that the setscrew heads in the cell body are facing up.

8) Unscrew the three #4-40 x 5/8-in. socket head screws which secure the cell retaining rings to the cell and body, and remove the ring that is facing up.

9) Remove the three spring disk washers and note that the direction of the disk contour alternates from disk to disk. When the cell is reassembled, the disks must be replaced in the same alternating order.

10) Remove the window holder.

11) Remove the quartz window.

12) Remove the PTFE window seal that may be stuck to either the window or to the counterbore in the window holder.

13) Turn the cell body over.

14) Remove the other retaining ring and repeat steps 9, 10 and 12.

15) Wipe the cell window with a soft cotton cloth soaked in isopropanol, methanol or acetone, or other suitable solvent. If the window is scratched or cannot be cleaned, replace it with the appropriate part. The 1.4 µl cell has a lens (0271-1479) in the side of the flowcell which faces the rear of the detector when the cell is in its kinematic mount. The face of the lens with the greatest curvature is positioned so that it is on the outside surface of the cell body.

16) Reassemble the cell with new PTFE seals. Make sure the contour of the disk washers alternates from washer to washer. Place the first washer in the recess with the outer rim down and the curved portion facing up.

17) Flush out the reassembled cell with distilled water, or other suitable solvent.

18) Place the sample cell back in the detector and tighten the large thumbscrew (see item 6, Figure 4-2) on top of the cell.

19) If disconnected, reconnect the inlet and outlet tubing.

20) Close the detector cover and replace the securing screw and cap.

4.7 DETECTOR PURGE

4.7.1 Optical Compartment

Depending upon the type of environment and wavelength selected, it may be necessary to purge the optical compartment to remove residual UV-absorbing vapors. Only pre-purified nitrogen should be used. Alternatively an effective moisture and particulate scrubber must be installed in the line from the tank to the detector. See item 6, Figure 4-9 to locate the purge hose fitting. Use a 10 cm (4-in.) length of 1/16-in. stainless steel tubing.

1) Carefully form a right-angle bend in the tubing so that both legs are approximately 5 cm (2") long. (See Figure 4-10.)

2) Insert one leg into the hose fitting and slide 17 gauge Teflon tubing over the other end of the stainless steel tubing. (Teflon tubing is supplied as P-E Part No. 0087-3140.)

3) Use appropriate fittings to connect the nitrogen line to the Teflon tubing.

   Important: Use only stainless steel tubing or fluorocarbon (Teflon) tubing in the nitrogen line. Do not use rubber or any other types of plastic tubing.

4) Nitrogen regulated at 5 psi must be clean and dry. Preferably use boiled-off liquid nitrogen.

Optical purge should be complete after 2 hours.

4.7.2 Cell Purge

From time to time it will be necessary to purge the flow cell. Proceed as follows.

1) Remove the column from the system, and bypass it with clean tubing. Use 0.015-in. I.D. tubing from the bypass tubing (or dummy column) to the detector inlet.

2) Pump isopropanol through the system for five minutes. Use a flow of 6 ml/minute for the 8 µl flow cell. Use a flow rate of 1 ml/min. for the 1.4 µl flow cell.

3) Shut off the pump.

4) Disconnect the pump outlet from the detector inlet and connect the nitrogen supply to the LC-85B inlet.

   Important: Use only stainless steel tubing and fluorocarbon (Teflon) tubing for the nitrogen line. Do not use rubber tubing or any other plastic tubing.

5) Employ a slight nitrogen purge (40 psi for the 8 µl cell, 60-80 psi for the 1.4 µl cell) to flush the cell. Use clean, dry, and preferably pre-purified, nitrogen for the purge.

6) Purge should be complete after 20 minutes.

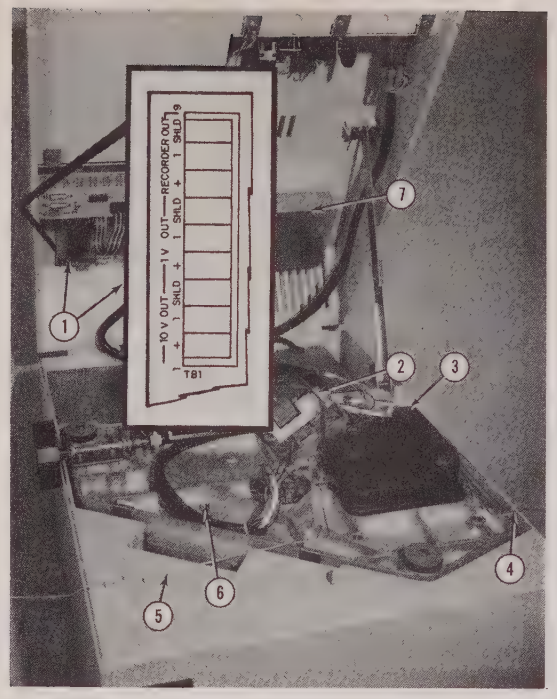


Figure 4-9 - LC-85B (Shown with Cover Lifted)

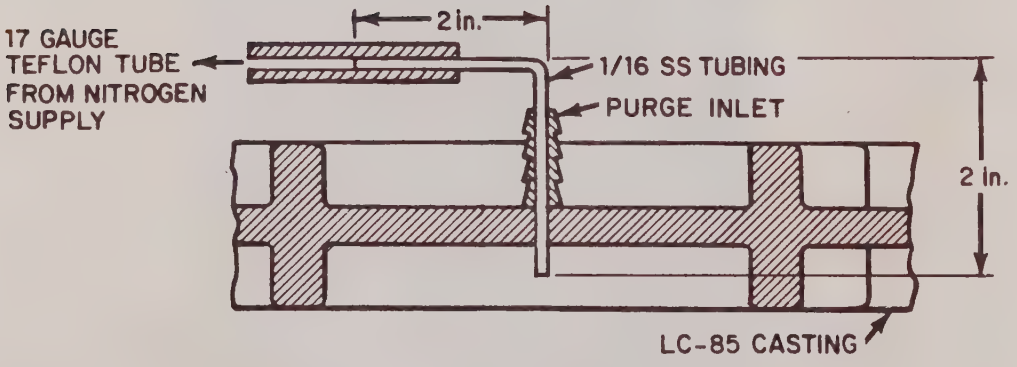


Figure 4-10 - Nitrogen Purge Fitting, Cross-sectional View

4.8 FLOW CELL RE-INSTALLATION

After replacing the flowcell peform the alignment procedures of Sections 4.8 or 4.9 whenever the lamp has been removed or replaced, or when changing from a 8 microliter cell to a 1.4 microliter cell or vice-versa.

> **Warning**: Never look directly at the deuterium lamp when it is on. Always wear ultraviolet opaque safety glasses whenever the cover is lifted and the lamp is on to avoid UV damage to the eyes. The lamp is hot. Allow the lamp to cool completely or wear protective gloves to avoid burns when handling the lamp.

It is not necessary to realign the cell when the flow cell alone has been removed, and the same cell replaced in the LC-85B.

1) Place the flow cell in the support plate and tighten the knurled thrumb screw of the flowcell into the casting.

2) Connect the inlet and outlet tubing to the flow cell.

3a) On the 8 µl flowcell, secure the lines with the knurled thumb screws on either side of the support plate (see Figure 4-11a).

3b) On the 1.4 µl flowcell, tighten the inlet and outlet lines into the unions on the L-shaped bracket on the casting (see Figure 4-11b).

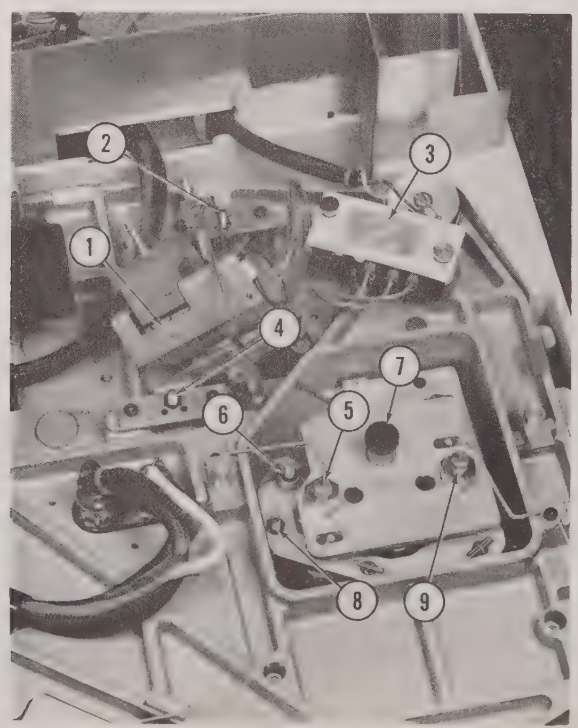


Figure 4-11a - 8 µl Flowcell Solvent Lines

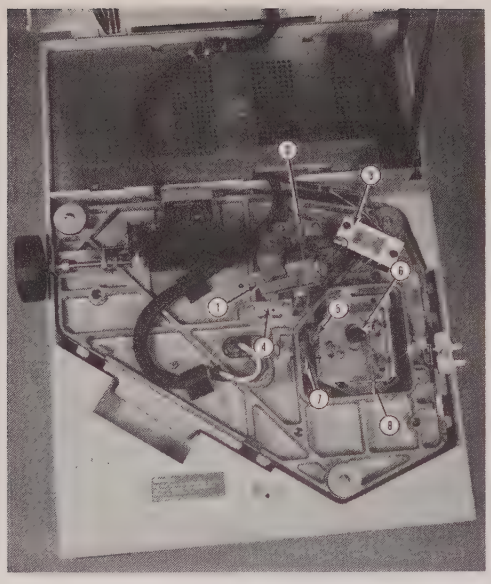


Figure 4-11b - 1.4 µl Flowcell Solvent Lines

4.9 ALIGNING THE 8 µl FLOWCELL

1) Rotate the horizontal adjustment screw exactly 5 complete rotations in the clockwise direction. (Note: This is a reversal of the rotation of step (6) in the procedure (2.7) for adjusting the Deuterium Lamp.)

2) Rotate the cell horizontal rotation (item 7, Figure 4-2) and the cell vertical tilt (item 5, Figure 4-2) adjustment screws alternately until a minimum absorbance indication is obtained. This is a maximum deflection in the baseline direction. It may go to a negative voltage.

3) Replace the cell adjust cover with the operation cover removed in step (5) of the Lamp Adjustment procedure (2.7).

4.10 ALIGNING THE 1.4 µl FLOWCELL

1) Rotate the horizontal adjustment screw exactly 5 complete rotations in the clockwise direction. (Note: This is a reversal of the rotation of step (6) in the procedure for adjusting the Deuterium Lamp.)

2) Rotate the cell horizontal rotation (item 7, Figure 4-2) and the cell vertical tilt (item 5, Figure 4-2) adjustment screws alternately until a minimum absorbance indication is obtained. This is a maximum deflection in the baseline direction. It may go to a negative voltage.

3) Adjust the focus travel screw (item 8, Figure 4-2) for a maximum voltage in the negative direction.

4) Replace the cell adjust cover with the operation cover removed in step 5) of the Lamp Adjustment procedure (2.7).

4.11 ENERGY CHECK

This procedure will provide an indication of whether or not satisfactory energy from the deuterium source is reaching the photo-tubes. In the range of 190 to 600 nm the ultraviolet energy emitted by the lamp will always be less at a wavelength of 190 nm. Therefore, if energy at this wavelength is sufficient to balance a 10 mV recorder signal it is a good indication that the lamp is in satisfactory condition and it and is in correct alignment with the flowcell.

1) This test should always be performed with a clean and dry flowcell.

2) With the POWER switch on for both the LC-85B and the recorder, allow the lamp to warm up for approximately 15 minutes.

3) Ascertain that the recorder is adjusted for a 10 mV full scale signal. Zero the recorder using the ZERO switch (short input leads). With the input shorted use the recorder zero control to bring the pen on scale at about 10% of full scale. Open the short on the input leads.

4) Set the wavelength of the LC-85B to 190 nm.

5) Using the LC-85B ZERO control move the recorder pen to the recorder zero baseline.

   If insufficient energy exists to offset the recorder pen it is suggested that the lamp be changed and/or its alignment with the flowcell be checked.

4.12 WAVELENGTH CALIBRATION CHECK

1) Switch on the POWER and allow at least 30 minutes stabilization time. Make certain that the recorder is operating with the Attenuator switch at 0.16 A_{1cm}.

2) Lift the detector cover.

3) Remove the cell cover and replace it with the cell-adjust cover, supplied.

4) Turn the cell horizontal screw (item 7 in Figure 4-2) counterclockwise until the cell blocks the sample beam as indicated by a maximum absorbance indication on the recorder (maximum downscale pen deflection).

5) Remove jumper SP6, located on the filter board (Figure 4-12), from the AB position and put it in BC position. The recorder pen will go upscale.

6) Zero the recorder.

7) Turn the Wavelength Control Knob for an indication on the WAVELENGTH indicator of 3 nanometers below 0 (zero) nanometers.

8) Scale to zero and beyond and record the wavelength at which peak energy (maximum absorbance = maximum downscale recorder deflection) occurs. It should be 0 ±1.2 nm.

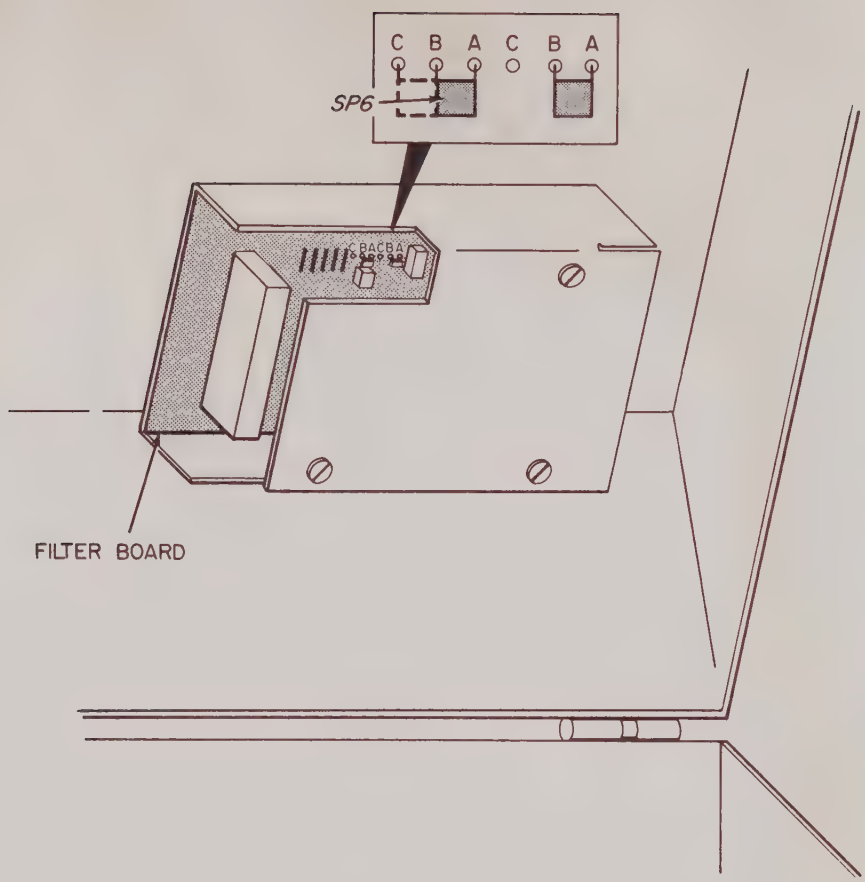


Figure 4-12 - Jumper SP6 and the Filter Board

9) Turn the Wavelength Control Knob for an indication of 421 nm on the WAVELENGTH indicator.

10) Scan from 421 to 425 nanometers. An emission band (downscale deflection of recorder pen) should occur at 423.1 ±1.0 nm.

11) If the peak is not observed in either case, or if the wavelength at which the peak energy is observed deviates from the above, repeat the procedure. If the deviation continues to occur, call a Perkin-Elmer Service Engineer.

12) Replace SP6 in position AB (see Step 7, above).

13) Turn the Wavelength Control Knob for an indication of 241 on the WAVELENGTH indicator.

14) Turn the cell horizontal adjust screw (item 7, in Figure 4-2) for maximum energy (minimum absorbance = maximum upscale recorder pen deflection).

15) Replace the cell-adjust cell cover with the cell cover removed in step 5, above.

SECTION 5
SPECIFICATIONS

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5.2	Optical	5-1
5.3	Electrical	5-1
5.4	Cell	5-2

5 SPECIFICATIONS

5.1 GENERAL

Power Requirements:	75W (max.) at 117V, 60 Hz (nominal) and at 230V, 50 Hz (nominal)
Ambient Temperature Range (Operating within specification):	19°C to 30°C
Size:	43.4 cm (17 1/16 in.) wide x 29.8 cm (11 3/4 in.) high x 58.4 cm (23 in.) deep
Weight:	27.2 kg (61 lbs.)

5.2 OPTICAL

Wavelength Range:	190 to 600 nm
Wavelength Accuracy:	±1.0 nm (approach from low side)
Wavelength Repeatability:	±0.3 nm (approach from low side) (manual operation)
Source Lamp:	deuterium
Wavelength Set:	manual control knob
Wavelength Display:	mechanical; readable to 0.1 nm
Order Filter:	Automatic change at 350 and 550 nm nominal
Linearity Range:	Linear within ±0.1% from 0 to 2 A_{1cm} at 275 nm. Within ±5% from 0 to 3 A_{1cm} at 275 nm.
Absorbance Accuracy:	Better than 1.0% from 0 to 1.5 A_{1cm} at 275 nm. Total dynamic range, min. 4.0 A_{1cm} at 208 nm.
Purge:	Optical paths enclosed are purgeable with clean dry nitrogen for use in contaminated environments.

5.3 ELECTRICAL

Theoretical Spectral Bandwidth:	2.9 nm at 250 nm. 2.6 nm at 600 nm.
Recorder Sensitivity Range Select:	11-postion binary push-button switch. Sensitivity 0.005 A_{1cm} to 5.12 A_{1cm} full scale with 10 mV recorder.

Zero Set (at recorder output only).	Ten-turn potentiometer fine zero.
Baseline Stability:	0.0010 A_{1cm} unit per hour (max.) After 1 hour warm-up period at 240 nm. Clean dry 8 µl cell.
Noise:	0.0002 A_{1cm} unit peak-to-peak (max.) at 240 nm; clean dry 8 µl cell.
Response Time Select:	Three position switch - 20 ms, 200 ms, 2000 ms. 3 pole Bessel function active filters.

Output

Recorder:	10 mV for each $Absorbance_{1cm}$ range
Data Output:	2 volt/per 1 $Absorbance_{1cm}$ (10V output) 200 mV/per $Absorbance_{1cm}$ range (1V output)

5.4 CELL

Volume:	8 microliters standard; 1.4 microliters optional
Maximum Pressure:	28 MPa (4000 psi); 7 MPa (1000 psi)
Path Length:	6 mm electronically normalized to standard A_{1cm} response.

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